Genetics and Molecular Biology, 35, 2, 545-552 (2012)
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Research Article

The complete mitochondrial genome sequence of the black-capped capuchin (*Cebus apella*)

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Abstract

The phylogenetic relationships of primates have been extensively investigated, but key issues remain unresolved. Complete mitochondrial genome (mitogenome) data have many advantages in phylogenetic analyses, but such data are available for only 46 primate species. In this work, we determined the complete mitogenome sequence of the black-capped capuchin (*Cebus apella*). The genome was 16,538 bp in size and consisted of 13 protein-coding genes, 22 tRNAs, two rRNAs and a control region. The genome organization, nucleotide composition and codon usage did not differ significantly from those of other primates. The control region contained several distinct repeat motifs, including a putative termination-associated sequence (TAS) and several conserved sequence blocks (CSB-F, E, D, C, B and 1). Among the protein-coding genes, the *COII* gene had lower nonsynonymous and synonymous substitutions rates while the *ATP8* and *ND4* genes had higher rates. A phylogenetic analysis using Maximum likelihood and Bayesian methods and the complete mitogenome data for platyrrhine species confirmed the basal position of the Callicebinae and the sister relationship between Atelinae and Cebidae, as well as the sister relationship between Aotinae (*Aotus*) and Cebinae (*Cebus*/*Saimiri*) in Cebidae. These conclusions agreed with the most recent molecular phylogenetic investigations on primates. This work provides a framework for the use of complete mitogenome information in phylogenetic analyses of the Platyrrhini and primates in general.

Key words: Cebus apella, control region, mitochondrial genome, phylogenomics.

Received: September 10, 2011; Accepted: March 1, 2012.

Introduction

The phylogenetic analysis of primates has attracted much attention because of the potential for defining and understanding the processes that mold, shape and transform the human genome. Primate taxonomy based on morphological, adaptive, bio-geographical, reproductive and behavioral traits, with inferences from the fossil record (Goodman *et al.*, 1998; Groves, 2001; Wilson and Reeder, 2005), is complex. Molecular genetic data have been used to infer many relationships among primate taxa (Hayasaka *et al.*, 1988; Schneider *et al.*, 1993; Moreira, 2002; Opazo *et al.*, 2006; Hodgson *et al.*, 2009; Wildman *et al.*, 2009; Perelman *et al.*, 2011). The phylogenetic relationships of Neotropical primates (Platyrrhini) in the suborder Haplorrhini are not as well understood as those of their closest relatives, the Old World monkeys and apes (Catarrhini) (Stewart and Disotell, 1998; Raaum *et al.*, 2005). The monophyly of three major lineages (Atelidae, Cebidae and Pitheciidae) within the platyrrhines has been confirmed, but the relationships among these three groups have been difficult to resolve (Horovitz *et al.*, 1998; Steiper and Ruvolo, 2003; Ray *et al.*, 2005; Opazo *et al.*, 2006; Schrago, 2007). The most recent studies support a basal division of the Pitheciidae and a sister relationship between Atelidae and Cebidae (Hodgson *et al.*, 2009; Perelman *et al.*, 2011). The relationships among different genera in the Atelidae and Pitheciidae are well-established (Opazo *et al.*, 2006; Wildman *et al.*, 2009; Perelman *et al.*, 2011) whereas the results for the Cebidae are more controversial, and various possible relationships among the Aotinae, Callitrichinae and Cebinae have been inferred (Kay, 1990; Schneider *et al.*, 1993; Moreira, 2002; Opazo *et al.*, 2006; Hodgson *et al.*, 2009; Wildman *et al.*, 2009; Perelman *et al.*, 2011). The most recent studies suggest a sister relationship between the Aotinae and Callitrichinae and a sister relationship between Aotinae/Callitrichinae and Cebinae (*Cebus*/*Saimiri*) (Hodgson *et al.*, 2009; Perelman *et al.*, 2011). The taxonomy of the genus *Cebus* is controversial, with different opinions on the classification of species within this group (Mittermeier and Coimbra-Filho, 1981; Groves, 2001; Silva Júnior, 2002); the phylogenetic relationships among species also remain unresolved, despite molecular and cytogenetic studies of this issue (Moreira, 2002; Amaral *et al.*, 2008; Garcia-Cruz *et al.*, 2011; Nieves *et al.*, 2011; Perelman *et al.*, 2011).

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In molecular systematics, the topology structures of the phylogenetic trees vary with the molecular markers used and the number of taxa involved. A comparison of related data from different taxa can be helpful in clarifying controversial topologies. In recent years, the mitochondrial genome (mitogenome) has been widely used in phylogenetic studies because of its matrilineal inheritance, lack of extensive recombination and accelerated nucleotide substitution rates (Ingman *et al.*, 2000; Zhang *et al.*, 2008). A complete mitogenome contains more information on the evolutionary history of a species than individual genes, and the use of this genome in phylogenetic analyses reduces stochastic errors and minimizes the effect of homoplasy (Campbell and Lapointe, 2011). Mitogenomes can also be used as a source of molecular markers in conservation studies of endangered species (Krajewski *et al.*, 2010; Meyer *et al.*, 2011). To date, the complete mitogenomes of 46 primate species have been sequenced, including five platyrrhine species (*Aotus lemurinus*, *Ateles belzebuth*, *Callicebus donacophilus*, *Cebus albifrons* and *Saimiri sciureus*) (Arnason *et al.*, 2000; Hodgson *et al.*, 2009).

Cebus apella (Linnaeus, 1758), the tufted or black-capped capuchin occurs only in South America (Colombia, Ecuador, Peru, Bolivia, Brazil, French Guiana, Suriname and Venezuela) (Fragaszy *et al.*, 2004). *C. apella* is under severe pressure from hunting and habitat loss and fragmentation throughout its range, with a sharp decline in numbers in recent years; the species has been included in CITES-Appendix II since the 1970s (Nijman *et al.*, 2011).

Although several molecular phylogenetic and cytogenetic studies of *C. apella* have been reported (Moreira, 2002; Amaral *et al.*, 2008; Wildman *et al.*, 2009; Nieves *et al.*, 2011; Perelman *et al.*, 2011), the complete mitogenome sequence of this species has not yet been described. In this work, we determined the complete nucleotide sequence of the *C. apella* mitogenome and determined its genomic structure. We also compared the rates of nonsynonymous (Kn) and synonymous (Ks) substitutions in protein-coding genes (PCGs) with those of *Homo sapiens* and five other platyrrhine species. A phylogenomic tree that included six platyrrhine species confirmed the usefulness of complete mitogenome sequences for molecular phylogenetic investigations.

Materials and Methods

Tissue samples and genomic DNA extraction

Samples from one specimen of *C. apella* were collected from Kunming Zoo, Yunnan Province, China. The specimen was identified based on external characteristics, using the system of Groves (2001) and Nowak *et al.* (1999). Total genomic DNA was isolated from fresh muscle samples by using a Wizard Genomic DNA purification kit (Promega) according to the manufacturer's instructions.

Mitochondrial DNA amplification by PCR

The mitogenome was amplified by the polymerase chain reaction (PCR) technique. The entire mitogenome of *C. apella* was obtained by using 12 primer sets to amplify contiguous and overlapping segments (Table 1) (Sorenson *et al.*, 1999; Ingman *et al.*, 2000). All fragment sequences overlapped each other by at least 200 bp. PCR amplifications were done in a Mycycler Gradient thermocycler using a final volume of 50 μL that contained 20-50 ng of genomic DNA (0.5 μL), 2.5 mM of each dNTP (4 μL), 10 μM of each primer (1 μL), 5 μL of 10x buffer, 0.25 μL of *Taq* polymerase (5 U/μL; Takara) and 38.3 μL of sterile distilled water. The cycling parameters were: preliminary denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 45 s, annealing at 55 °C for 45 s, elongation at 72 °C for 2 min, with a final elongation at 72 °C for 10 min. The PCR products were electrophoresed on a 1.5% agarose gel and visualized by ultraviolet transillumination after staining with ethidium bromide. A negative control was included in each round of PCR to

Table 1 - Primer pairs used in the PCR amplifications.

Primer	Sequence (5'-3')	Primer	Sequence (5'-3')
mtDNA1H	CTG GTT GAT CCT GCC AGT	mtDNA1L	AGA CTC CTT GGT CCG TGT TTC AAG AC
mtDNA2H	AAA CTG GGA TTA GAT ACC CCA CTA T	mtDNA2L	ATG TTT TTG GTA AAC AGG CG
mtDNA3H	GGA GAT AAG TCG TAA CAA GGT AAG CA	mtDNA3L	TCC TAC GAT GTT GGG TCC TTT
mtDNA4H	AAT CCA GGT CGG TTT CTA TCT A	mtDNA4L	ATC CTA TAT GGG CGA TTG ATG AGT
mtDNA5H	CGA AAA TGT TGG TTT ATA CCC TTC C	mtDNA5L	TGC CAA GCT CTG TGG TGA AT
mtDNA6H	GGA CTG CAA GAA CAT ATC TCA CAT CAA	mtDNA6L	GGA GGA GGA CAT CCA TGT AGT CAT TC
mtDNA7H	GCT CAT TTA TTT CAC TAA CAG CAG T	mtDNA7L	GGG CTA CAG CAA ATT CAA GGA T
mtDNA8H	TTG GCT CAC TTT CTA CCT CAA GG	mtDNA8L	GTG GGG ATG ATG ATT TTT AGC ATT GTA
mtDNA9H	CCA AAA CAA ATG ATT TCG ACT CA	mtDNA9L	GGT TCC TAA GAC CAA TGG ATT ACT TCT
mtDNA10H	TCA CTC TCA CTG CCC AAG AA	mtDNA10L	AGA AGG TTA TAA TTC CTA CG
mtDNA11H	GCT GAG ARG GHG TDG GMA TYA TRT C	mtDNA11L	CCT CAG AAK GAT ATY
mtDNA12H	CTA ACA TGA ATC GGA GGA CAA CCA G	mtDNA12L	GGC TCA TCT AGG CAT TTT CAG TG

check for contamination. The PCR products were stored at 4 °C until purification and sequencing.

Sequence assembly, annotation and analysis

The DNASTAR software package (Lasergene version 5.0; Madison, WI) was used for sequence assembly and annotation. The borders of PCGs and rRNA genes were determined by aligning the sequences with those of *C. albifrons* (AJ309866) and *H. sapiens* (NC_001807) in GenBank. The boundaries and orientations of tRNAs were identified by tRNAscan-SE version 1.21 under default settings. The rates of Kn and Ks in PCGs were calculated using PAML version 4 (Yang, 2007). Pairwise distances of selected taxa were inferred from mitochondrial DNA (mtDNA) by MEGA version 5 (Tamura *et al.*, 2011). The complete nucleotide sequence was submitted to GenBank under accession No. JN380205.

Phylogenomic inference using mitogenome

The complete mitogenomes of *C. apella* and five other platyrrhine species available from GenBank (*Aotus lemurinus*, FJ785421; *Ateles belzebuth*, FJ785422; *Callicebus donacophilus*, FJ785423; *Cebus albifrons*, AJ309866; *Saimiri sciureus*, FJ785425) were used to examine the phylogenomic relationships among these species, with *Pan troglodytes* (NC_001643) and *Homo sapiens* (NC_001807) used as outgroups.

Phylogenomic analysis was done with a Maximum likelihood (ML) method implemented in MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001) and PhyML 3.0 (Guindon *et al.*, 2010). The best fitting model of sequence evolution was obtained by using Modeltest version 3.7 (Posada and Crandall, 1998). The GTR+I+G model was selected as the model of best fit. For the Bayesian procedure, four independent MCMC chains were simultaneously run for 5,000,000 replicates by sampling one tree per 1000 replicates. We discarded the first 1250 trees as part of a burn-in procedure and used the remaining 3750 sampling trees (of which log likelihoods converged to stable values) to construct a 50% majority rule consensus tree. Two independent runs were used to provide additional confirmation of the convergence of the posterior probability distribution. In the ML analysis, a BIONJ tree was used as a starting tree to search for the ML tree with the GTR+I+G model. The robustness of the phylogenetic results was tested by bootstrap analysis with 1000 replicates.

Results and Discussion

Genome organization and composition

The complete mitogenome of *C. apella* is a circular molecule with 16,538 bp (Figure 1). This size is intermediate to that of other primate mitogenomes (Matsui *et al.*, 2009; Matsudaira and Ishida, 2010; Roos *et al.*, 2011), with the longest being 17,149 bp (*Eulemur macaco*) (Matsui *et al.*, 2009) and the shortest being 15,467 bp (*Pygathrix nemaeus*) (Roos *et al.*, 2011). The number and arrangement of genes were the same as those of other primate mitogenomes (Boore, 1999). Table 2 shows the various features of the genome, together with the inferred start and stop codons. The complete mitogenome consisted of two rRNAs, 22 tRNAs, 13 PCGs and a control region. As expected, two rRNAs, 14 tRNAs and 12 PCGs were encoded on the H-strand, while the *ND6* gene and eight tRNAs were encoded on the L-strand. Most of the PCGs were separated by one or more tRNAs.

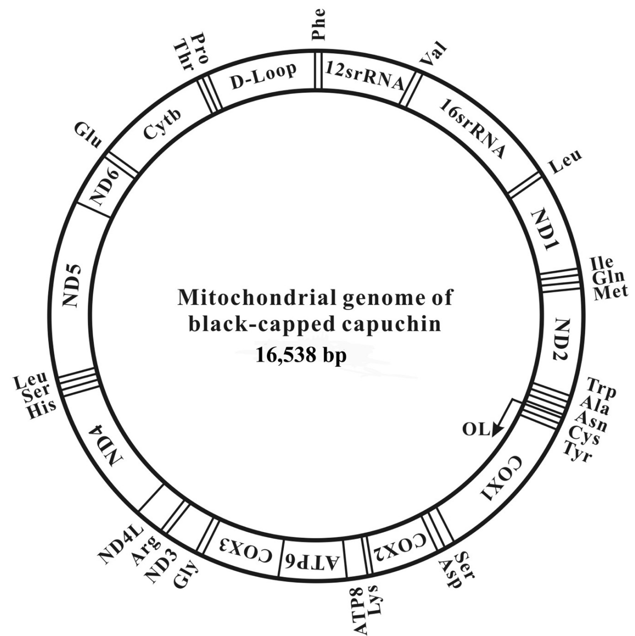

Figure 1 - Gene organization of *C. apella* mitochondrial DNA. *ND1-6* refers to NADH dehydrogenase subunits 1-6, *COI-III* refers to cytochrome c oxidase subunits 1-3, *ATP6* and *ATP8* refer to ATPase subunits 6 and 8, and Cyt *b* refers to cytochrome b.

The nucleotide composition of the *C. apella* mitogenome was biased towards adenine (A) and thymine (T), and the overall A+T content was 60.6%. There were seven regions in which genes overlapped by 70 bp and 15 intergenic spacer regions that were 57 bp in size.

Protein coding genes and codon usage

There were no significant differences in the sizes of the *C. apella* PCGs compared to other primates (Arnason *et al.*, 1996). Most of the 13 PCGs began with an ATG start codon, except for *ND2* and *ND3* (ATT), *COII* and *ND4L* (GTG) and *ND6* (TTA). Six PCGs (*ND1* (TA), *COII*, *COIII*, *ND2*, *ND3* and *ND4* (T)) did not terminate with a complete stop codon triplet (Table 2). In mammalian mitogenomes, some peptide-coding genes end with T or TA rather than with a complete stop codon; in such cases, the terminal nt is contiguous with the 5' terminal nt of a tRNA gene (Wolstenholmde, 1992; Arnason *et al.*, 1996) and post-transcriptional polyadenylation can modify the in-

Table 2 - Organization of the *C. apella* mitochondrial genome.

Gene	Position[a]		Size (bp)	Spacer (+)/ overlap (-)	Strand[b]	Codon	
	From	To				Start[c]	Stop[c]
tRNA-Phe	1	70	70		H		
12s-rRNA	71	1030	960		H		
tRNA-Val	1031	1088	58		H		
16s-rRNA	1089	2651	1563		H		
tRNA-Leu (UUR)	2652	2726	75	2	H		
ND1	2729	3684	956		H	ATG	TA[d]
tRNA-Ile	3685	3753	69	-3	H		
tRNA-Gln	3751	3822	72	3	L		
tRNA-Met	3826	3890	65	2	H		
ND2	3893	4931	1039		H	ATT	T[d]
tRNA-Trp	4932	4998	67	8	H		
tRNA-Ala	5007	5074	68	1	L		
tRNA-Asn	5076	5148	73	2	L		
OL	5151	5184	34	-2	L		
tRNA-Cys	5183	5249	67	-1	L		
tRNA-Tyr	5249	5317	69	1	L		
COI	5319	6875	1557	-13	H	ATG	AGG
tRNA-Ser(UCN)	6863	6931	69	3	L		
tRNA-Asp	6935	7002	68		H		
COII	7003	7690	688		H	GTG	T[d]
tRNA-Lys	7691	7755	65		H		
ATP8	7756	7956	201	-40	H	ATG	TAA
ATP6	7917	8597	681	24	H	ATG	TAA
COIII	8622	9405	784		H	ATG	T[d]
tRNA-Gly	9406	9472	67		H		
ND3	9473	9818	346	1	H	ATT	T[d]
tRNA-Arg	9820	9888	69	1	H		
ND4L	9890	10186	297	-7	H	GTG	TAA
ND4	10180	11554	1375		H	ATG	T [d]
tRNA-His	11555	11623	69		H		
tRNA-Ser(AGY)	11624	11682	59		H		
tRNA-Leu(CUN)	11683	11753	71	2	H		
ND5	11756	13561	1806	-4	H	ATG	TAA
ND6	13558	14091	534		L	TTA	TAA
tRNA-Glu	14092	14160	69	4	L		
Cyt *b*	14165	15301	1137	2	H	ATG	TAA
tRNA-Thr	15304	15373	70	1	H		
tRNA-Pro	15375	15442	68		L		
Control region	15443	16538	1096		H		

[a]Position numbering starts with the 5' position of tRNA-Phe; [b]Genes transcripted from the L or H strand; [c]Start and stop codons of protein coding genes; [d]Protein coding genes overlapping with tRNA genes end with an incomplete stop codon.

complete stop codon to a complete one (Ojala *et al.*, 1981; Boore, 2004).

There were 3788 codons in the 13 mitochondrial PCGs, excluding incomplete termination codons. A bias towards a greater proportion of A and T is a common feature of primate mitogenomes and results in a corresponding bias in the encoded amino acids (Arnason *et al.*, 1996; Yu *et al.*, 2011). The overall AT composition of PCGs in *C. apella* was 60.9% and the most frequently used amino acids were Leu (12.67%), Ile (10.32%), Ser (9.35%) and Thr (8.98%).

Ribosomal and transfer RNA genes

Like other primate mitogenomes, the *C. apella* genome contained small (12S) and large (16S) subunits of

rRNA (Yu *et al.*, 2011). The 12S rRNA and 16S rRNA were located between the tRNA-Phe and tRNA-Leu (UUR) genes, separated by the tRNA-Val gene. The base compositions of the two rRNA genes were 23.8% T, 23.6% C, 35.7% A and 16.9% G, which generally agreed with the A+T-rich trend of the whole genome.

Twenty-two tRNA genes were identified based on their respective anticodons and secondary structures, and ranged in size from 58 (tRNA-Val) to 96 (tRNA-Ser) nucleotides. All of the tRNAs folded into a canonical cloverleaf secondary structures, except for tRNA-Phe, tRNA-Met, tRNA-Thr, tRNA-Lys and tRNA-Trp. Gene sizes and anticodon nucleotides agreed with those described for other primates. However, G-G, A-C, and especially G-U wobbles and other atypical pairings were identified in the stem regions. These mutations appear to accumulate in mitochondrial genes, partly because mtDNA is not subject to recombinations that would facilitate the elimination of deleterious mutations (Li *et al.*, 2007). The postulated tRNA cloverleaf structures generally contained 7 bp in the aminoacyl stem, 5 bp in the T-stem and the anticodon stem, and 4 bp in the D-stem. Some tRNAs, *e.g.*, tRNA-Val and tRNA-Gly, lacked one or two bp in the T-stem or anticodon stem.

Non-coding regions

The major non-coding region, *i.e.*, the control region, of *C. apella* was located between the tRNA-Pro and tRNA-Phe genes and contained 1,096 bp. This region can be divided into three domains based on the distribution of variable nucleotide positions and the differential frequencies of nucleotides (Wang *et al.*, 2008). The control region shows marked variability across taxonomic groups and among related species, but its sequence elements related to regulatory functions are highly conserved (Cui *et al.*, 2007). We annotated the regulatory domains of *C. apella*, *H. sapiens* and five other platyrrhine species (Figure 2). Termination-associated sequence (TAS) motifs that act as a signal to terminate synthesis of the control region were found at the 5' end of the first domain. There were five conserved sequence boxes (F, E, D, C and B) in the second domain. The third domain had a conserved sequence block (CSB-1) that is important in regulating mtDNA replication. There was less similarity between the sequences of *H. sapiens* and platyrrhine species.

The small non-coding region, a putative origin of light strand replication (O_L), was located in a cluster of tRNA-Trp, tRNA-Ala, tRNA-Asn, tRNA-Cys, and tRNA-Tyr (the WANCY region) and consisted of 34 nucleotides. This region could potentially fold into a stable stem-loop secondary structure with 10 bp in the stem and 14 bp in the loop. The conserved motif 5'-GCCCC-3' at the base of the stem within the tRNA-Cys gene has been associated with the transition from RNA synthesis to DNA synthesis.

Mitochondrial DNA variations in five platyrrhine species

The relative influence of natural selection can be determined by comparing the rates of Kn versus Ks in the coding region of protein genes (Jiang *et al.*, 2009). The *ND6* gene is generally excluded in such analyses because it is encoded by a different strand of the mtDNA and has a strikingly different nucleotide composition relative to other mitochondrial PCGs (Arnason *et al.*, 1996). As shown in Table 4, the Kn/Ks ratios for all pairwise combinations varied among the 12 mtDNA PCGs, suggesting that differential selective constraints have acted on these parameters (Cui *et al.*, 2007). The Kn/ Ks ratios of the *ND1* and *ATP8* synthase genes were higher than those of other genes, especially in the *ATP8* synthase gene (Table 3). This finding suggests that the *ND1* and *ATP8* synthase genes may have evolved faster than the other 12 protein-coding genes. Sur-

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                        91      TAS        112  406      CSB-F      428   440    CSB-E     461
Cebus apella            TACATTATTGTTTATCCCCATG  CTC--CGTGAAACCACCAACCCGCC  CTCTTCTCGCTCCGGGCCCATA
Aotus lemurinus         .G.....A..C...G.......  ...............G.........  ......................
Ateles belzebuth        .G........C.CG........  ...............A.........  G.A.C.................
Callicebus donacophilus .......A..C..TA.......  ...............T..TT..AT.  .C.C..CTTGC.G.C.......
Cebus albifrons         .G........C...........  .........................  ......................
Saimiri sciureus        .G.....A......G.......  ...............G.........  ......................
Homo sapiens            ATT.G..G.A.GGGAGTGGGA.  T.GAA...AGGTG.GAT..ATAATA  GAA.CAAA.ACAGATA.TGCG.

                        491 CSB-D   506 549    CSB-C     569 623 CSB-B  637 786    CSB-1      806
Cebus apella            ATCTGGCATTTGGTTC TTAAATAAGACATC-ACGAGGG GGGGAAAGGGGGGGG GTCAATGGTTTCAGGACATAA
Aotus lemurinus         .........C...... ...................T.. TA...TTTTT..... ............G........
Ateles belzebuth        .........C...... ...................T.. .....GTTAT.C.A. A.T......CA..........
Callicebus donacophilus .AAC.....C...... ......G......TTGT..T.. T..CCT.CT...TA. .........CA..........
Cebus albifrons         .C.............. ...................T.. ............... .........C...........
Saimiri sciureus        ................ ...................T.. ............... ....................G
Homo sapiens            CAA..CT..CGC..G. G.G.G.GGTTA..-..G.GT.A TA..CTTTAT..--- -..C....GGA.GA..AGGG.
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Figure 2 - Sequences and the conserved elements of the control region. Primary sequence features (TAS, CSB-B-F, CSB-1) are shown in boxes. CSB – conserved sequence block, TAS – termination-associated sequence.

prisingly, the *COIII* gene had a higher mutation rate than the *COI* and *COII* genes in *C. apella*; this is not present in other platyrrhine species (Table 3).

Phylogenomic relationships of six platyrrhine species

Mitochondrial sequences can be used not only to infer phylogenetic relationships and directly trace the evolution of gene rearrangements, but also to provide additional information for phylogenetic reconstructions (Braband *et al.*, 2010). The molecular divergence and separation time of two *C. apella* populations have recently been investigated based on Cyt *b* sequence data (Casado *et al.*, 2010); this study illustrates the value of mitochondrial sequences in ecological and conservation studies of primates. In contrast to the use of isolated or individual sequences, *e.g.*, the Cyt *b* sequence, recent molecular studies have tended to use greater larger amounts of DNA data since this allows better tree resolution and provides better agreement with morphological studies (Zhang and Wake, 2009).

A phylogenetic analysis based on the complete mitogenome data from six platyrrhine species (*A. lemurinus*, *A. belzebuth*, *C. donacophilus*, *C. albifrons*, *C. apella*, and *S. sciureus*) in conjunction with ML and Bayesian methods supported the sister relationship between *C. apella* and *C. albifrons* (100%) and the sister relationship between the Aotinae (*Aotus*) and Cebinae (*Cebus*/*Saimiri*) (Figure 3). The results also confirmed the basal position of the Callicebidae and the sister relationship between the Atelinae and Cebidae (Aotinae/Cebinae). These findings were supported by pairwise distance analysis in which the distance between *C. apella* and *C. albifrons* was the closest while that between *C. apella* and *C. donacophilus* was the greatest, except for the outgroups (Table 4).

Our results generally agreed with the conclusions of recent molecular phylogenetic work on primates (Hodgson *et al.*, 2009; Perelman *et al.*, 2011), although only a few species were included in our tree and the resulting phylogenetic information was too limited. More complete mitogenome data are urgently needed to investigate the

Table 3 - Rates of nonsynonymous (Kn) and synonymous (Ks) substitutions in the coding region of protein genes between *C. apella* (CA) and other selected species.

		ND1	*ND2*	*COI*	*COII*	*ATP8*	*ATP6*	*COIII*	*ND3*	*ND4L*	*ND4*	*ND5*	Cyt*b*
CA *vs. Aotus lemurinus*	Kn	0.1006	0.0374	0.0696	0.0247	0.2739	0.0788	0.1137	0.1030	0.2065	0.1011	0.0876	0.0974
	Ks	0.4646	0.5329	1.7221	0.7899	0.9370	1.7521	0.5318	0.7523	1.1656	0.7157	0.7056	0.7364
	Kn/Ks	0.2165	0.0702	0.0404	0.0313	0.2923	0.0450	0.2138	0.1369	0.1772	0.1413	0.1241	0.1323
CA *vs. Ateles belzebuth*	Kn	0.0834	0.0638	0.0650	0.0219	0.1307	0.0791	0.1183	0.0913	0.2330	0.1244	0.0883	0.1033
	Ks	0.4783	0.2887	1.1512	0.8163	0.8265	0.7954	0.5712	0.9008	1.1331	0.6564	0.7272	0.8174
	Kn/Ks	0.1744	0.2210	0.0565	0.0268	0.1581	0.0994	0.2071	0.1014	0.2056	0.1895	0.1214	0.1264
CA *vs. Callicebus donacophilus*	Kn	0.1233	0.1129	0.0655	0.0239	0.2278	0.0668	0.1096	0.1284	0.2362	0.1261	0.1173	0.0994
	Ks	0.5766	0.4131	1.5320	1.0386	1.6108	1.5649	0.5616	0.9902	1.2670	0.7773	0.9081	0.7466
	Kn/Ks	0.2138	0.2733	0.0428	0.0230	0.1414	0.0427	0.1952	0.1297	0.1864	0.1622	0.1292	0.1331
CA *vs. Cebus albifron*	Kn	0.0543	0.0123	0.0645	0.0114	0.0845	0.0326	0.0596	0.0731	0.2642	0.0114	0.0359	0.0333
	Ks	0.2169	0.2871	0.7228	0.2723	0.3057	0.2850	0.1887	0.9300	1.0462	0.0158	0.2918	0.1476
	Kn/Ks	0.2503	0.0428	0.0892	0.0419	0.2764	0.1144	0.3158	0.0786	0.2525	0.7215	0.1230	0.2256
CA *vs. Saimiri sciureus*	Kn	0.1069	0.0636	0.0751	0.0314	0.4131	0.1044	0.1039	0.1408	0.2630	0.1346	0.0980	0.1016
	Ks	0.5351	0.6005	1.2448	0.6911	0.6818	1.0097	0.5786	0.9967	2.3726	0.6817	0.7731	0.7969
	Kn/Ks	0.1998	0.1059	0.0603	0.0454	0.6059	0.1034	0.1796	0.1413	0.1108	0.1974	0.1268	0.1275

Table 4 - Pairwise distances of selected platyrrhine species inferred from the mitochondrial genome.

	1	2	3	4	5	6	7	8
1. *Cebus albifrons*								
2. *Cebus apella*	0.100							
3. *Saimiri sciureus*	0.213	0.225						
4. *Ateles belzebuth*	0.199	0.210	0.217					
5. *Callicebus donacophilus*	0.223	0.237	0.239	0.210				
6. *Aotus lemurinus*	0.189	0.203	0.201	0.175	0.211			
7. *Homo sapiens*	0.320	0.326	0.323	0.309	0.325	0.314		
8. *Pan troglodytes*	0.320	0.325	0.322	0.305	0.324	0.311	0.096	

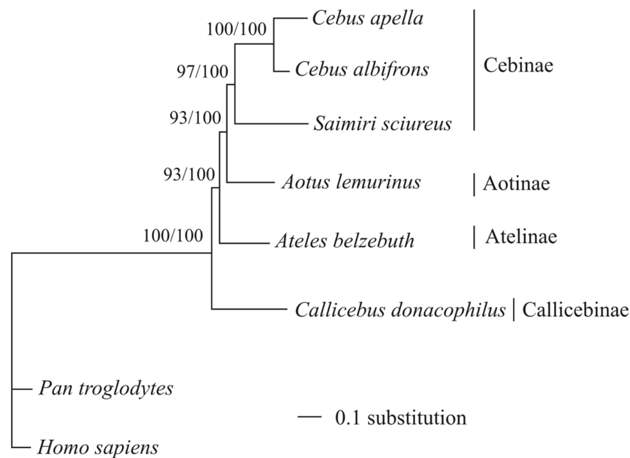

Figure 3 - Maximum likelihood (ML) and Bayesian tree based on complete mitochondrial genome with the GTR+I+G model. The horizontal length of each branch corresponds to the substitution rates estimated with the model. *Pan troglodytes* and *Homo sapiens* were used as outgroups. Numbers on the branches are bootstrap values for ML and Bayesian posterior probability.

phylogeny of the Cebidae, Platyrrhini and primates in general. The sequence data presented here provide a contribution to this long-term goal.

Acknowledgments

We thank Kunming Zoo for providing the tissue samples of *C. apella*. This work was supported by the Natural Scientific Foundation of China (grants 30800605 and 31171189) and the Natural Scientific Foundation of Shandong Province, China (grant ZR2009DZ003).

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Associate Editor: Fabrício Rodrigues dos Santos

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